



# Comparative Histological Evaluation of Effect of Night Guard Vital Bleaching with Three Different Concentrations of Carbamide Peroxide (10%, 16% And 22%) with Amorphous Calcium Phosphate on Dental Pulp – An In Vivo Study”

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## Abstract:

**Aims and Objectives:** To histologically evaluate the effect of Night Guard Vital Bleaching gel with three different concentrations of carbamide peroxide (10%, 16% And 22%) with Amorphous Calcium Phosphate. (ACP) on dental pulp.

**Material and Methods:** 18 patients participated in the study with an age criteria of 15-25 years. Patients divided into three groups. Group A patients were treated with 10% carbamide peroxide with ACP. Group B and Group C with 16% and 22% carbamide peroxide with ACP respectively. Each group was subdivided into four sub groups according to days for application of bleaching gel i.e., 4 days, 14 days, 14 days followed by 14 days recovery and control group. Impression was taken and bleaching trays were fabricated.

**Results:** Group A and Group C showed statistically significant difference while Group B with Group A and Group C were not statistically significant.



**Conclusion:** The results were encouraging as the inflammatory reactions were reversible in the recovery period with no dentinal sensitivity in any of the patients within the groups.

**Key Words:** bleaching, carbamide peroxide, amorphous calcium phosphate.

**DOI Number:** 10.48047/nq.2022.20.22.NQ10366

**NeuroQuantology 2022;20(22):3683-3691**

### Introduction:

Esthetic dentistry has become an exciting new frontier in our profession. Aesthetics, by definition, is the science of the beauty, that particular detail of an animate or inanimate object that makes it appealing to the eye (Arens, 1989).<sup>[1,2]</sup>

The newer treatment procedures are conservative, painless and more reliable and contribute towards better esthetic outcomes. In this context tooth bleaching has gained enormous popularity.<sup>[3]</sup> Bleaching is simplest, least invasive and least expensive means available to lighten discolored teeth.

Carbamide peroxide a new bleaching agent were introduced for treatment of discolored tooth, e.g. at various concentrations that is, 3% to 45%.<sup>[5, 6, 7]</sup> Carbamide peroxide readily decomposes to urea and hydrogen peroxide in saliva. Hydrogen peroxide breaks down into oxygen and water, which then penetrate the tooth and liberate the pigment molecules (Haywood & Heymann<sup>[8,9]</sup>, 1992).

The scientific literature is lacking on the deleterious effects on the pulp and other safety issues of carbamide peroxide whitening gel greater than 10% concentrations that is 16% and 22% carbamide peroxide whitening gel ACP (Nite White).

### Aims and Objectives:

The aim of the present study was to evaluate and compare the histological changes in the pulp in response to night guard vital bleaching procedure using three different concentration of carbamide peroxide that is 10%, 16% and 22% carbamide peroxide with ACP (Nite White Excel 3) for time period of 4 days, 14 days and 14 days treatment followed by 14 days recovery period.

Following are the objectives of the study:-

- 1) To evaluate histologically the effect of 10% carbamide peroxide gel with ACP on the dental pulp for time period of 4 days,

- 14 days and 14 days treatment followed by 14 days recovery period.
- 2) To evaluate histologically the effect of 16% carbamide peroxide gel with ACP on the dental pulp for time period of 4 days, 14 days and 14 days treatment followed by 14 days recovery period.
- 3) To evaluate histologically the effect of 22% carbamide peroxide gel with ACP on the dental pulp for time period of 4 days, 14 days and 14 days treatment followed by 14 days recovery period.
- 4) To compare the effect of 10%, 16% and 22% carbamide peroxide gel with ACP on the dental pulp.
- 5) To compare the effect of 10%, 16% and 22% carbamide peroxide gel with ACP on the dental pulp with the control group (no treatment).

### Materials and Methods:

#### Patient inclusion criteria:

- Permanent maxillary and mandibular first premolars scheduled for orthodontic extraction.
- Completed root formation which was confirmed by radiograph.
- 15 to 25 years of age.

#### Tooth inclusion criteria:

- Absence of caries.
- Absence of restorations.
- Absence of visible structural defects.
- Absence of pulpal symptoms or radiographic periapical lesions.

The study population consisted of 18 patients, with a mean age of 20 years, requiring the extraction of permanent maxillary and mandibular premolars for orthodontic reasons. Before commencement of the study, patient was explained about the procedure. The patients who



agreed to be a part of the study were asked to sign a consent form.

#### **First appointment-**

After complete oral prophylaxis of patients, maxillary and mandibular alginate impressions were taken. Study models were made and reservoirs were made on the facial surface of teeth #5, #12 and #21.

#### **Fabrication of Bleaching Tray-**

Vacuum-formed bleaching trays were then fabricated from 0.035-inch polyvinyl sheets (Sof-Tray, Ultradent Products Inc.) for every of the maxillary and mandibular casts with the help of vacuum machine (Easy-Vac, Medes, Co.).

The study population was divided into three groups of 6 patients each which are as follows:

**Group A:** Patients were treated with 10% carbamide peroxide containing amorphous calcium phosphate.

**Group B:** Patients were treated with 16% carbamide peroxide containing amorphous calcium phosphate.

**Group C:** Patients were treated with 22% carbamide peroxide containing amorphous calcium phosphate.

Each group (Group A, Group B, and Group C) was divided into four sub groups:

**Subgroup 1.** #5 (maxillary right first premolar) was treated with carbamide peroxide with ACP (Nite White Excel 3) for 4 days before extraction.

**Subgroup 2.** #12 (maxillary left first premolar) was treated with carbamide peroxide with ACP for 14 days before extraction.

**Subgroup 3.** #21 (mandibular left first premolar) was treated with carbamide peroxide with ACP for 14 days; same tooth was kept for 14 days without treatment before extraction.

**Subgroup 4.** #28 (mandibular right first premolar), which was not treated with any bleaching material (control group).

#### **Second appointment –**

The patient was provided a mandibular bleaching tray with a single reservoir for tooth #21, one tube of dual barrel syringe of 10% carbamide peroxide with ACP whitening gel and a container to hold the tray when not in use. The

tray was placed in the mouth to ensure a good fit with no interference. The patient was instructed to wear the tray loaded with 10% carbamide peroxide with ACP in reference to #21 for at least six hours each night for 14 days.

#### **Third appointment-**

- It is scheduled on 15<sup>th</sup> day after the second appointment. Mandibular bleaching tray was taken and then the maxillary bleaching tray was given with reservoirs for teeth #12 and #5, along with one tube of whitening gel. They were then instructed to bleach the tooth #12 for 14 days as described above; and on the 11th day, they have to begin with bleaching of tooth #5 for last four days.

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The patients were questioned about any sensitivity during the bleaching treatment during daily examination.

The above mentioned same protocol for application of bleaching gel with respect to time was followed for 16% and 22% carbamide peroxide gel with amorphous calcium phosphate respectively.

#### **Extraction -**

The teeth were atraumatically extracted under local anesthesia (2% lidocaine with 1:100,000 epinephrine). Immediately after extraction, the teeth were wiped clean of any debris with gauze.

#### **Preparation of the tooth for histological evaluation-**

The most apical 4 mm of the root was than sectioned off using diamond disk. After sectioning, the tooth was placed in an air tight bottle containing 10% neutral buffered formalin. Each bottle was labeled with a patient identification number and the corresponding tooth number. All the samples were processed and given for histological processing in pathology lab. The teeth were demineralized in 5% HNO<sub>3</sub> for 25-30 days. The teeth were then embedded in paraffin and serially sectioned axio-facio-lingually through the pulp. Slides were made and then stained with Hematoxylin and eosin stain.

#### **Histological Evaluation-**

Leica (DML) Research microscope with Monitor was used at 40 x to 400x magnification for examination of the pulp tissue in stained sectioned teeth.

The evaluation included:

- The presence of inflammatory cells and
- Irregularities in the odontoblastic layer,

The inflammatory reaction of pulp in response to night guard vital bleaching with 10%, 16% and 22% carbamide peroxide with Amorphous Calcium Phosphate (ACP) was categorized according to number of inflammatory cells present on the histological section of the pulp (**Browne, Plant, Tobias<sup>5</sup>1980**).

Score 0 = no inflammatory cells  
(Absence of inflammation)

Score 1 = 5-25 inflammatory cells  
(Mild inflammation)

Score 2 = 26-100 inflammatory cells  
(Moderate inflammation)

Score 3 = >100 inflammatory cells  
(Severe inflammation)

Score 4 = Abscess.

All results obtained were noted & statistical analysis was done.

#### Statistical Analysis:

Statistical analysis to compare the histological changes in the pulp in response to night guard vital bleaching procedure using three different concentration of carbamide peroxide i.e. 10%, 16% and 22% carbamide peroxide with ACP was done using following test.

The statistical tests used for the analysis of the results were:

- 1) Tukey multiple comparison test
- 2) Students unpaired t-test
- 3) Chisquare test
- 4) Intra class correlation

**Graph 1:** Comparison of Subgroup 1 (4 days) of all the Groups according to scores given by three observers

**Graph 2:** Comparison of Subgroup 2 ( 2 weeks) of all the Groups according to scores given by three observers

**Graph 3:** Comparison of Subgroup 3 (2 weeks+2 weeks recovery) of all the Groups according to scores given by three observers.

#### RESULTS

The comparison between Group A (Fig .4 ) and Group B(Fig .5 )showed non significant ( $p>0.05$ ) results. It suggests that both the groups are exhibiting statistically similar inflammatory reactions in dental pulp.

The comparison between Group B and Group C (Fig .6)showed non significant ( $p>0.05$ ) results. It suggests that both the groups are causing statistically similar type of inflammatory reactions in dental pulp.

The comparison between Group A to Group C showed highly significant ( $p<0.05$ ) results. It suggests that Group C exhibited highly significant inflammatory scores as compared to Group A.

When Subgroup 1, Subgroup 2 and Subgroup 3 of Group A, Group B and Group C were compared with each other for different period of time interval the results were statistically significant between Subgroups of Group A and Subgroups of Group C. While Subgroups of Group A and Subgroups of Group B showed non significant results. Similarly Subgroups of Group B and Subgroups of Group C showed non significant results.

None of the teeth demonstrated a severe reaction to the Carbamide Peroxide with ACP. None of the patients had experienced any pulpal discomfort or dentinal sensitivity during treatment.

#### Discussion:

Nightguard vital bleaching (NGVB), or “matrix bleaching” or “dentist-prescribed/home-applied bleaching” has received much attention as an effective and simple method for lightening stained or discolored teeth. <sup>[12,13,14,15]</sup> Since **Haywood and Heymann<sup>[17]</sup>** first reported this technique in **1989**, several studies have documented its success and effectiveness.

Young patients were selected for the present study because they had intact teeth that were subject to minimal wear or age changes. The enamel and dentin in teeth from young patients is also more permeable than that of older individuals (*Mjör, Sveen & Heyeraas*<sup>[18]</sup> 2001).

The time intervals selected for bleaching in the present study were based on previous studies by *Cohen and Chase*<sup>[19]</sup> (1979), *Fugaro & Mjör*<sup>[20]</sup> (2004), where four days had been the shortest time to show histological changes and two weeks was generally considered the minimum time recommended by dentists and manufacturers for use of carbamide peroxide bleaching agents. .

For present study, grading/scoring system was used as the standardized method for evaluating the inflammation. This was in accordance with the previous studies in which the degree of tissue response is graded by *Browne and Plant 1980 et al*<sup>[21]</sup>.

None of the patient in all the three groups, Group A Group B and Group C reported sensitivity during the period of treatment (access during daily records). This may be due to the presence of ACP which helps in precipitation of its crystalline molecules (from the bleaching gel) in the tubules that reduces the permeability to an extent that may prevent any adverse reaction in the pulp (*Mjör and Ferari*<sup>[22]</sup>2000).

While comparing **Group A** with **Group B** statistically non significant histologic reaction in the pulp was found. It suggests that both the groups showed inflammatory reaction to same extent in dental pulp after bleaching for the same period of interval.

While comparing **Group B** with **Group C** statistically non significant histologic reactions in the pulp were noted. It suggests that both the groups showed inflammatory reaction to same extent in dental pulp after bleaching for the same period of interval.

While comparing **Group A** with **Group C**, Group C exhibited statistically significant ( $p < 0.05$ ) inflammatory scores. This may be attributed to the higher concentration of the carbamide peroxide resulting in the faster penetration of the

hydrogen peroxide in the dentinal tubules and more will be the pulpal reaction.<sup>[22, 23]</sup>

This was in accordance with the study by *Bowles and Ugwuneri*<sup>[24]</sup> (1987) and *Cooper and colleagues*<sup>[25]</sup>(1992) who described that the diffusion is a concentration-dependent phenomenon (that is, the higher the concentration, the faster the diffusion and more will be the inflammatory changes).

When **Subgroup 3** (14 days treatment+14days without treatment) of all the three groups were compared **Subgroup 3** of **Group A** showed maximum recovery from inflammation when compared with the **Subgroup 3** of **Group B**, and **Group C**. But Subgroup3 of all the Groups (A, B and C) were statistically non significant from each other suggesting recovery of pulp after recovery/rest period.

The above mentioned results obtained were in accordance with the study by *Fugaro JO, Fugaro N, Matis BA ,and Mjör IA*<sup>[26]</sup> (2004) they have shown that the night guard vital bleaching of teeth with 10% carbamide peroxide gel may cause minor short-term reactions in pulp (in 4 days and 14 days). If such changes occur, they are reversible over time (14 days rest/recovery period). Hence Subgroup 3 showed no evidence of inflammation.

The **Subgroup 4** of all the groups that is Group A, Group B and Group C which served as control group (they were not treated with any bleaching material) showed normal histological appearance of the dental pulp.

The few artifacts found in the present study which were ignored like vacuolization in odontoblastic layer ,the presence of red blood cells outside blood vessels, fibrosis in the body and irregular odontoblastic layer.

These results are encouraging for Night Guard Vital Bleaching with 10%, 16% and 22% carbamide peroxide with amorphous calcium phosphate as they caused mild to moderate inflammatory reactions which were of reversible in nature.

## CONCLUSION

Based on the results obtained and discussed the following conclusions were drawn from the present study:-

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- 1) Histological evaluation of teeth treated with 10% carbamide peroxide with amorphous calcium phosphate for time period of :-
  - a) **4 days**- showed slight reactions (dilated blood vessels).
  - b) **14 days**- only three teeth showed mild inflammation, while three teeth showed no reaction.
  - c) **14 days treatment followed by 14 days without treatment** - none of the teeth showed inflammatory reaction which suggests that inflammation resolved in recovery period.
- 2) Histological evaluation of teeth treated with 16% carbamide peroxide with amorphous calcium phosphate gel for time period :-
  - a) **4 days**- showed mild inflammation in three teeth and the rest three teeth showed no reaction.
  - b) **14 days**- five teeth showed mild inflammation while one tooth showed no reaction.
  - c) **14 days treatment followed by 14 days without treatment**- None of the teeth showed inflammatory reaction which suggests that inflammation resolved in recovery period.
- 3) Histological evaluation of teeth treated with 22% carbamide peroxide with amorphous calcium phosphate gel for time period of
  - a) **4 days**- showed mild inflammation in six teeth.
  - b) **14 days**- two teeth showed moderate inflammation and the rest showed mild inflammation.
  - c) **14 days treatment followed by 14 days without treatment**- none of the teeth showed inflammatory reaction which suggests that inflammation resolved in recovery period.
- 4) When the histological effects of 10%, 16% and 22% carbamide peroxide with amorphous calcium phosphate bleaching agents were compared with each other, the inflammatory changes were significantly pronounced in 22% as

compared to 10% carbamide peroxide with ACP. While comparison between 10% and 16% carbamide peroxide with ACP and 16% and 22% carbamide peroxide with ACP have shown non significant changes in inflammatory reaction.

- 5) Nightguard vital bleaching with 10%, 16% and 22% carbamide peroxide with amorphous calcium phosphate have shown significant histologic pulp changes when compared with the control group (no treatment).

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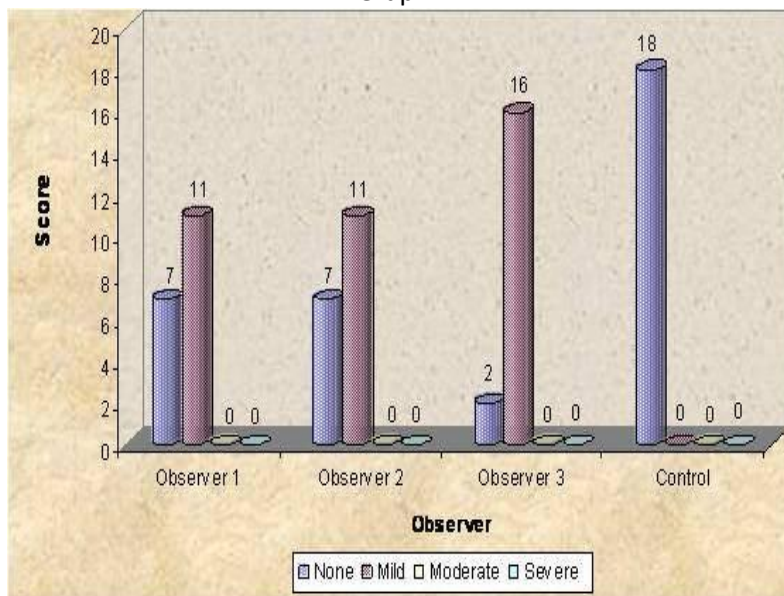


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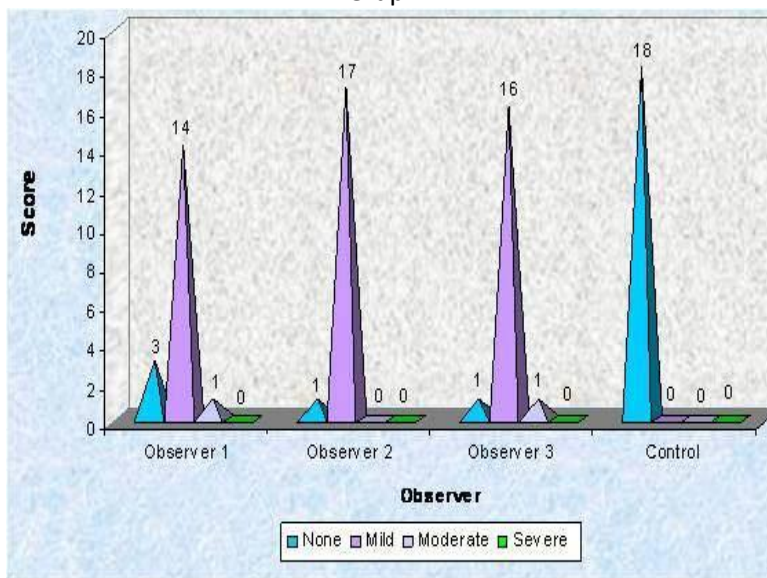


Graph 1



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Graph 2





Graph 3

